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CILIATE PARASITES OF FRESHWATER ORNAMENTAL FISH

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AUTHORS' CONTRIBUTIONS

All the authors made substantial contributions to the conception of the work. Author JG wrote the first draft of the manuscript. Authors AD and TD prepared the images. Authors FS and JG did the literature survey. All authors have contributed to the manuscript preparation, image analysis and approved its submission.

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ABSTRACT

The aquarium fish keeping began as a small hobby and now emerged as an international trade worth millions of dollars in the export market. Although India is in a marginal position, it has great potential in ornamental fish production due to its rich biodiversity of fish species. Export of ornamental fish from India mostly dependent on freshwater fish species, contributing nearly 80% of the total trade. Parasitic infection and diseases are one of the major obstacles limiting expansion and yield of the aquaculture industry. Ciliates are the most common parasites identified from skin, gills and fins of infected fish in aquculture farm. Important pathogenic ciliates like *Ichthyopthirius multifiliis*, *Chilodonella* sp., *Trichodina* sp., *Tetrahymena* sp. and *Epistylis* sp. cause significant morbidity and mortality in variety of fish species in artificial closed environments of aquaria. Some common genera are obligate parasites whereas few are commensal, facultative and opportunistic. Use of appropriate chemotherapeutic agents and suitable management strategies can prevent and reduce aquarium fish diseases. Current investigations showed that protective immune responses against many ciliate parasites develop in fish bodies. Hence, vaccines are suggested as the effective solution to tackle the growing concerns of pathogenic infections in fish by providing protection, health benefits as well as economic gain.

Keywords: *Ciliate parasites; Ornamental fish; morphology; pathology; prophylaxis.*

1. INTRODUCTION

There is a rapid development and growth in the ornamental fish trade in the International market for the last 40 years. But at the same time concern has been expressed on the overdependence of this trade on the natural wild population of freshwater and marine fish [1].

Although India is still in a marginal position, its trade is developing rapidly. India can become one of the leaders in the trade if it can utilize the diverse indigenous fish resources. In India, most of the vendors maintain fish without healthy and appropriate quarantine practices. Due to lack of suitable transportation facilities these fish are frequently becoming infected with various diseases and at the same time spreading it very fast causing epidemic. Therefore, disease is one of the major constraints and limiting factors of ornamental fish culture and should be addressed to prevent huge economic loss [2].

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Parasites commonly found in freshwater ornamental fishes are protozoa, helminthes and arthropods [3]. Host fish are mostly attacked by ciliate parasites like *Trichodina* sp., *Ichthyopthirius multifiliis*, *Chilodonella* sp., *Tetrahymena* sp. [4] and are frequently isolated in large numbers from skin, gills and internal organs. It has been found that ciliates and myxozoans are responsible for serious illness in fish among all types of parasites [5]. Direct life cycle and asexual reproductive mode are advantageous for the parasites to increase in number during crowding in high stocking density.

The ciliated protozoans belonging to Phylum Ciliophora are an advanced, complex and diverse group. They are fast moving due to the presence of cilia and infra ciliature systems. They are unique among other protozoans because of nuclear dimorphism. Many of them possess permanent cell mouth or cytostome and cytopharynx to procure nutrients in the form of algae, bacteria, fungi and even other protozoa. Most of them are free-living heterotrophs or symbiotic. However, a major fraction of ciliates have adopted a parasitic lifestyle in aquatic invertebrates and fishes [6, 7]. Among them *Ichthyophthirius multifiliis* (often termed 'ich') poses a significant threat to the aquaculture industry [8]. It is responsible for causing white spot disease in freshwater fish and inflicts considerable damage and economic loss to cultured fish of all kinds [9]. Due to low host specificity and broad host range this endoparasite has an added advantage of attacking fins, body surface and gill epithelium of a variety of fish species to procure nourishments [10].

Another unicellular ciliate group Trichodinids are disc shaped ecto commensal or parasitic and many species of this parasite have been described from India and abroad [11, 12, 13]. Little studied ciliated protozoa of genus *Chilodonella* sp. are potential pathogens of fish and associated with mild to acute tissue damages like epithelial hyperplasia, necrosis and hemorrhagic lesions which may be fatal for the cultured fish [14]. Body surface, gills and fins are also attacked by sedentary ciliates like *Ambiphyra* and *Apisoma* and stalked ciliates *Epistylis*. Many free-living ciliates like *Tetrahymena* sp. which feed on bacteria and organic debris in natural water have become facultative parasites of various ornamental fish species.

2. GENUS: *Ichthyophthirius multifiliis* FOUQUET, 1876

2.1 Morphology and Life Cycle

I. multifiliis Fouquet, 1876 is a largest known parasitic protozoan found on fishes. The parasite's life cycle is composed of three stages: trophonts, tomonts and theronts. Theront is a small, pear shaped free-swimming infective and mobile stage measuring 30 to 50 µm which attaches and penetrates host fish epidermis. This stage develops into a feeding stage known as Trophont or mature adult parasite. It is round and oval in shape. Size of these parasites is highly variable from 0.5 to 1.5 millimeters in diameter. Body ciliature is uniform and parasites show a kind of slow, rolling movement. A vestibule is visible in the apical end with sparse ciliature and uses this during feeding on the basal lamina. The shape of the macro nucleus was found to be variable in different stages of life cycle (Fig. 1a, b, c). Immature Trophont (Pear shaped) are found with illipsoidal macro nucleus (Fig. 1a). But the macronucleus is observed as a typical horseshoe shaped structure in mature forms only (Fig. 1c, 2a). The trophont stage is visible in the host as individual white spots on the body surface and in fins of the host. When the mature trophont leaves the fish host it transforms into a tomont and covers its body into a cystic covering to attach on available substrate. Tomonts further undergo cell division to differentiate into tomites and finally transform into theronts to complete the cycle [15] (Fig.2b).

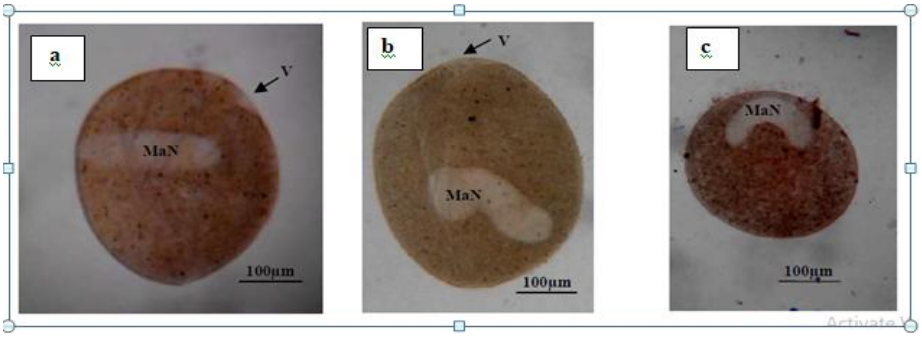

Fig. 1. Unstained *Ichthyophthirius multifiliis* a. Immature Trophont (Pear shaped) with illipsoidal macro nucleus. b. Trophont stage with sub apical vestibule (V) and Macronucleus (MaN). c. Mature Trophont with horse shoe shaped nucleus. [1000x magnification]

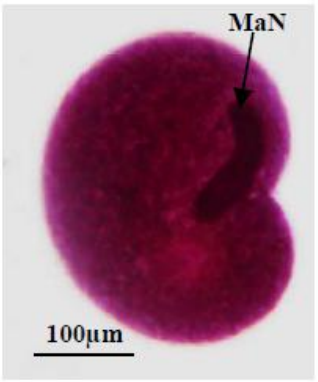

Fig. 2a. *Ichthyophthirius multifiliis*, Giemsa stained Trophont stage. [1000x magnification]

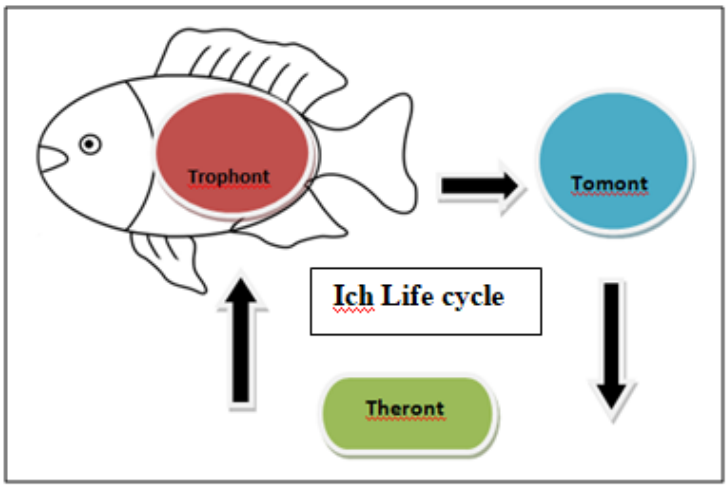

Fig. 2b. *Ichthyophthirius multifiliis* life cycle

2.2 Pathogenesis, Clinical sign and Treatment

Ich is a common and life threatening disease among cultured, wild ornamental fish and preferably occurs in relatively warm water. Temperature below 10^0C or above 28^0C can halt parasite growth [2]. The parasite feeds on the epidermal cells causing hyperplasia, desquamation and fin rotting. The parasite is macroscopically visible and the characteristic symptoms of the disease are small white spots over the body surface and gills, excessive slime production and rapid breathing. In high stocking density, respiratory suffocation and secondary bacterial infection may occur and result in mass mortality. Life cycle is dramatically affected by ambient water temperature. In summer parasites life cycle is short but lengthy during fall. *I. multifiliis* from carp fingerlings were reported by Banerjee and Bandyopadhyay in 2010 from West Bengal [16]. Severe infection of this disease was also recorded from goldfish, *Carassius auratus* during summer season in three districts of West Bengal, India [17]. Chanda et. al. (2011) showed that the intensity of infection of *Ichthyophthirius* sp. is high in summer months and low in winter. In a study performed in Korean fish farm, heavy infestation of *I. multifiliis* were reported from skins of three host species, Silver shark (*Balantiocheilos melanopterus*), Rainbow shark (*Epalzeorhynchos frenatum*) and Sumatra barb (*Puntitus tetrazona*) [2]. Frequent water change and combination of copper and formalin treatment can effectively control the parasite. Raising the aquaria temperature to 80^0F is a natural mode of parasite eradication [18].

3. GENUS: *Chilodonella* STRAND, 1928

3.1 Morphology and Life Cycle

Chilodonella are mostly free living. Although two species of *Chilodonella* are considered as important fish parasites namely *Chilodonella piscicola*

(Zacharias, 1894) Jankowski, 1980 [syn. *C. cyprini* (Moroff, 1902)] and C. *hexasticha* (Kiernik, 1909) Kahl, 1931 [19]. *Chilodonella hexasticha* prefers to stay in warmer climates temperature ranging between 26 and 31 °C, while *Chilodonella piscicola* having broader thermal tolerance between 4 and 20 °C. These ciliates are the most pathogenic species of fish cultured in freshwater [20]. Mitra and Haldar (2004b) for the first time described *C. hexasticha* from *Nandas nandus* (Hamilton Buchanan), a fresh water fish in India. Body shape of this parasite is oval and asymmetrical [21]. The apical end is narrow and with a characteristic notch. Observed *Chilodonella* specimens measured (30 - 65) µm in length and (20 – 50) µm in width. The cytostome is located anteriorly and opens through a median cytopharynx (Fig. 3a) and macro and micro nuclei are located at the posterior end (Fig. 3b). Body covered with parallel rows of cilia. These parasites, rather very transparent, attach with the ventral surface to the host body and reproduce by transverse binary fission. Occasionally they perform conjugation [22].

3.2 Pathogenesis, Clinical sign and Treatment

This parasite damage to the gill lamellae and enlargement of gill epithelial cells are common. The most frequent histopathological changes observed are hemorrhages, edema and necrosis of the host gill [14]. Symptoms of the infection are depigmentation of the body due to excessive mucus secretion, gill lesions and general weakness. Fish shows irritation and asphyxiation. The affected fish often come to the surface of the water to gasp air. C. *hexasticha* has been reported from *Symphysodon discus* in Japan in 1984. In 2013 *C. piscicola* were reported from *C. auratus* from Turkey [23]. It has been found that active penetration of the parasite cytostome inside the host body for feeding can result in an inflammatory reaction [20]. Limited information availability on the parasite host interaction, habitat and ecology of *Chilodonella* making its management more difficult. Preferred treatment for chilodonellosis is treating the infected fish in 1% soln. (5 ml per litre) of acriflavine. potassium permanganate, formalin and copper sulphate are also used to treat fish infected with *Chilodonella* spp.

4. GENUS: *Trichodina* EHRENBERG 1838

4.1 Morphology and Life Cycle

Trichodinids are ciliate that mostly act as ecto-commensal on fish and feed on suspended bacteria from the surrounding medium. But during heavy infection due to poor water quality and nutritional deficiency, it can cause severe epidermal lesion and damage of gill epithelium. Presently, there are more than two seventy genera described within the family Trichodinidae. Most common genera of the family include *Trichodina*, *Trichodonella*, and *Tripartiella*. The genus (*Trichodina* Ehrenberg 1838) is the largest of this family with more than 200 species described from fish [24]. Juvenile stages of the host fish are more susceptible to this disease in a polluted and oxygen poor environment [25].

This parasite shows high morphological variations in the structure of adhesive discs which is very important taxonomically. Four known species of *Trichodina* namely *T.chittogongensis* Asmat and Mohammad (2005), *T. pseudominta* Tang and Zhao (2013), *T. kaptaiensis* Asmat and Mohammad (2005), *T. jialgensis* Tang and Zhao (2013) have been isolated and identified for the first time from Oranda gold fishes (*Carassius auratus auratus*) in India [26, 27].

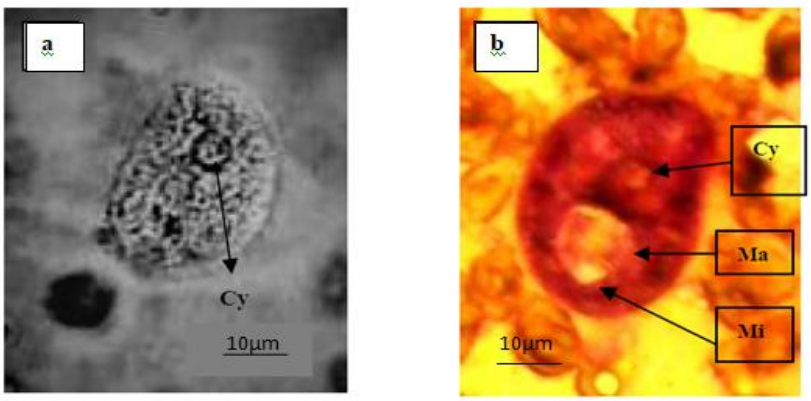

Fig. 3. *Chilodonella hexasticha* unstained (a) and Giemsa stained specimen (b). Cy. Opening of cytopharynx [400x magnification]

This parasite is the largest within the family Trichodinidae Raabe, 1959. Diagnosis of the parasite can be made in wet mount preparation. This is a round, saucer-shaped ciliate. Adoral surface is supported by a ring of cytoskeletal denticles and attaches with the host body. When viewed from top, the parasite resembles a disc-shaped structure and from the side view resembles a saucer (Fig. 4a). The disc is commonly called the adhesive disc. There are three rings of cilia covering the body (Fig. 4b).

4.2 Pathogenesis, Clinical Sign and Treatment

The parasite normally does not cause much harm or disease manifestation when present in low numbers. But in high stocking density it produces distinct lesions, induces epidermal cell hyperplasia and mucus production in the host. Juvenile and fingerlings are most susceptible and show ulceration, oedema of subepithelial tissue and displacement of secondary gill lamellae. Preferred treatment for *Trichodina* is bathing the infected fish in 2-3% salt solution for 2-5 min for 3-5 days [28]. *Trichodina* spp. infected Tilapia when treated with formalin it reduced the loads of *Flavobacterium columnare*, a Gram-negative bacterium causing columnaris disease [11].

5. GENUS: *Tetrahymena* FURGASON, 1940

5.1 Morphology and life Cycle

Tetrahymena is a saprozoic ciliate of the phylum Ciliophora frequently found on dead organic matter. This parasite is common to aquaria water and can infect skin, gills, fins and finally internal organs as secondary invaders. So far, about ten *Tetrahymena* species have been identified as pathogenic in diverse groups of organism namely *T. corlissi*, *T. pyriformis*, *T. stegomyiae*, *T. chironomi*, *T. vorax*, *T. rostrata*, *T. limacis*, *T. rotunda*, *T. glochidiophila* and *T. papula* [28]. It can infect many species of ornamental fish like cichlids, black mollies, and tetras although guppies (*Poecilia reticulata*) are more susceptible to infection and they are naturally infected by *Tetrahymena*. Body is oval (approx. 100 µm x 30 µm in size) and the anterior the portion is slightly tapering. *T. pyriformis* has on its right side an undulating membrane and buccal cavity bears a narrow pocket [29].

5.2 Pathogenesis, Clinical sign and Treatment

Infected fish develop necrotic skin lesions that gradually spread into the musculature, circulation and finally reach internal organs. Symptoms are visible as whitish lesions on the body surface and in affected tissue [28]. The parasite erodes the epidermis and stimulates strong inflammatory reactions. The disease was named as tet disease or Tetrahymeniosis. Treatment strategies of this parasitic infection are somewhat similar to ichthyophthiriasis [29].

6. GENUS *Epistylis* EHRENBERG, 1830

6.1 Morphology and Life Cycle

Epistylis are stalked, sessile, ciliated protozoans that are considered as emerging pathogens of ornamental fish. They are peritrich ciliates and love to inhabit water with high organic material and feed on bacteria in the water column. But occasionally, they attach with fish skin, gill and fin surface and even on fish eggs. Specifically, the bottom-dwelling fish and goldfish were the most sensitive group affected by this parasite. Parasitic crustaceans sometimes act as carriers for *Epistylis* spp. and transmit this ciliate to fish body and subsequently contaminate the aquarium [30]. These ciliates form colonial zooids and attach with the substrate by a non contractile stalk. When their peristome was expanded it forms peristomial folds [31].

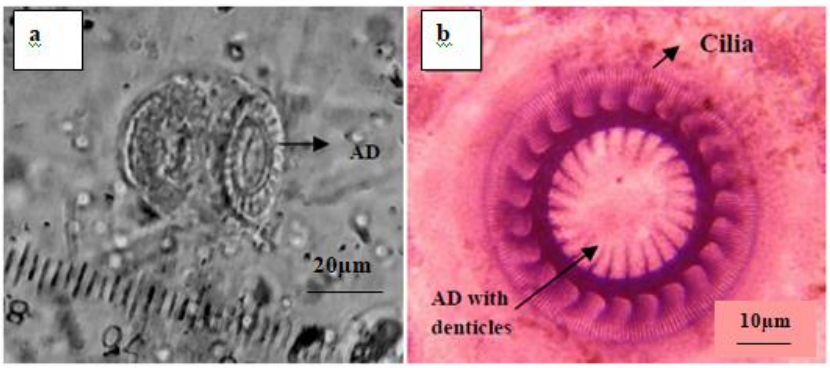

Fig. 4. Unstained *Trichodina* sp. in lateral view (a) and Giemsa stained specimen with central adhesive disc [400 x magnifications] (b). AD Adhesive Disc [1000x magnification]

6.2 Pathogenesis, Clinical sign and Treatment

Epistylis sp. is commonly associated with Gram-negative bacteria and causes "red sore" disease in fish. These two pathogenic agents in combination often produce haemorrhagic lesions, oedema and ulceration in fish. The infected host suffers from scale loss and erosion of fin spines. This parasite was reported from wild and cultured tilapia kept in high stocking densities [30]. Effective treatments recommended to cure these infections are salt baths and application of antiparasitic agents like formaldehyde 37%.

7. MOLECULAR DIAGNOSTIC TECHNIQUES FOR DETECTION OF PATHOGENIC CILIATES

Conventional pathogen detection methods in aquaculture are mostly dependent on examination of Giemsa or haematoxylin or Silver nitrate impregnated smears under microscope. Histological and biochemical techniques for detection and growing pathogens in suitable culture media also lack rapid and accurate diagnosis. New tools for detection and identification of ciliate parasites using sensitive gene specific technologies are necessary to be used in a large scale to reduce the chances of pathogen transfer in ornamental fish trade. Environmental DNA (eDNA) is an emerging diagnostic tool having potential to improve our present method of parasite detection [32]. This DNA is naturally shed by organisms into their surrounding environment. DNA present in different life stages of the parasite can be detected easily using Polymerase chain Reaction (PCR), Real time PCR [33], LAMP (loop-mediated isothermal amplification [34]. The current challenge is the development of a multiplex assay where multiple pathogens can be detected and quantified in a single reaction.

8. CONCLUSION

Ciliate parasites cause significant damage to the body surface, gill and skin of host fish. Parasite infected fish also are prone to different secondary infections. Therefore proper attention and care should be taken to prevent such infections. Ciliate parasites are generally treated with sodium chloride (common salt), $KMnO_4$, formalin and copper sulphate. Adequate prophylactic management methods should be applied to reduce and prevent ciliate infection of the pet fishes. With the growing popularity of this industry there is also potential scope of transfer of unknown pathogens and microbial communities. To control the occurrence of disease outbreaks, maintenance of aquaria fish with a good water exchange system, use of clean and sterilized utensils to avoid cross contamination, supply of healthy protein rich food are key to prevent pathogen infiltration. Proper parasitic species identification and species specific treatment and management are important.

Most of the aquaria farm depends solely on chemical treatment for pathogen elimination. Chemotherapeutics applied by different aquarists are not always totally effective and even hazardous to the aquaria water. Persistent use of these chemicals and antibiotic drugs is leading to the emergence of drug resistance and harmful effects on the environment [35].

Recent studies suggest that immunoprophylaxis is a better option to control pathogenic infection. According to the work of Dickerson *et al*. 1984 *Tetrahymena pyriformis* can be used as a protective antigen against *Ichthyophthirius multifiliis* infection [36]. Live, killed, subunit and DNA vaccines have been tested against *I. multifiliis* infection in a variety of fish hosts [37]. Recently, to control white spot disease vaccination of the host with ich preparation were tested and protective responses were generated [38,39]. In *I. multifiliis* immune responses are directed against two stages namely the theront and trophont stage [40]. The DNA vaccine for *I. multifiliis* also has been tested in rainbow trout and channel catfish as a vaccine candidate but their efficacy were comparatively lower than live vaccines [41]. Majority of the vaccines used have approval from United States Department of Agriculture (USDA) and utilized conventional culture practice of the target pathogen [42]. If an engineered vaccine can be prepared in future that may have the scope to impart protective immunity against other pathogenic ciliates also. Lastly, compulsory quarantine practice during transfer or movement of ornamental fish will ensure disease prevention.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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